Analysis of 4-(Methylnitrosamino)-1-(3-pyridyl-*N*-β-D-glucopyranuronosyl)-1-butanolonium inner salt (NNAL-*N*-Gluc) in the Urine of Smokers and Smokeless Tobacco Users.

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The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a potent pulmonary carcinogen in rodents and is believed to play a significant role as a cause of lung cancer in smokers. NNK is metabolically converted to NNAL, which is also strongly carcinogenic. Previous studies demonstrate that NNAL is metabolized in part to 4-(methylnitrosamino)-1-(3-pyridyl)-1-(*O*-β-D-glucopyranuronosyl)butane (NNAL-*O*-Gluc), which has been identified in the urine of smokers and smokeless tobacco users. Nicotine as well as its principal metabolite cotinine are metabolized to pyridine-*N*-glucuronides, suggesting that NNAL-*N*-Gluc could be formed from NNAL. Indeed, Murphy et al have identified NNAL-*N*-Gluc as a metabolite of NNAL in human liver microsomes (Kuehl and Murphy, Proc. Amer. Assoc. Cancer Res. 42: 162 (2001). We developed a method to analyze human urine for NNAL, NNAL-*O*-Gluc, and NNAL-*N*-Gluc. Urine was applied to a C_{18} solid phase extraction cartridge. Elution with 10% methanol gave a fraction containing NNAL-*N*-Gluc and NNAL-*O*-Gluc. Elution with 50% methanol gave a fraction containing free NNAL. One portion of the 10% methanol fraction was treated with 1.0 N NaOH, 80 °C, 1h to release NNAL from NNAL-*N*-Gluc while the other was treated with β-glucuronidase, 37 °C, 16h to release NNAL from NNAL-*N*-Gluc plus NNAL-*O*-Gluc. The released NNAL was further purified by HPLC, silylated, and analyzed by gas chromatography with nitrosamine selective detection. The free NNAL from the 50% methanol fraction was similarly analyzed. In 10 smokers, levels of NNAL, NNAL-*N*-Gluc, and NNAL-*O*-Gluc

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were (pmol/ml ± S.D.) 0.46 ± 0.21, 0.32 ± 0.16, and 0.43 ± 0.34. In 10 smokeless tobacco users, the corresponding amounts (pmol/ml ± S.D.) were 1.48 ± 1.13, 0.59 ± 0.60, and 2.13 ± 2.55. Thus, in smokers, NNAL-*N*-Gluc represented 50.4 ± 24.7% of total NNAL-Gluc whereas the corresponding figure in smokeless tobacco users was 23.6 ± 12.4%. LC-MS evidence for the presence of NNAL-*N*-Gluc in human urine was also obtained. The results of this study demonstrate that NNAL-*N*-Gluc is a human urinary metabolite of NNK and suggest that smoking may induce N-glucuronidation of NNAL.

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